Fig. S1. Genetic strategy to generate Piezo2-ChR2 mice. (**A**) Constitutive Piezo2-ChR2$^+$ mice were generated by crossing Piezo2-GFP-IRES-Cre mice to Ai32 mice. Ai32 mice carry the ChR2(H134R)-EYFP cassette at the Gt(ROSA)26Sor locus, separated from its CAG promoter by a floxed stop sequence. (**B**) Postnatal expression of ChR2 in Piezo2-positive sensory neurons was achieved by intraperitoneal injection of P0-P2 Piezo2-GFP-IRES-Cre mouse pups with AAV8 carrying the CAG-Flex-ChR2-tdtomato construct. AAV8: adeno-associated virus serotype 8), GFP: Green fluorescent protein, IRES: internal ribosome entry site, CAG: Cytomegalovirus early enhancer/chicken beta actin promoter, ChR2: channelrhodopsin-2, EYFP: enhanced yellow fluorescent protein, WPRE: Woodchuck Hepatitis Virus Posttranslational regulatory element, and ITR: inverted terminal repeats. (**C**) Bar graph representing quantification of viral transduction efficiency in Piezo2-ChR2$^+$ mice. Data averaged across 3 mice. (**D**) Percent behavioral response (paw withdrawal, paw licking, paw guarding, flinching, jumping, and vocalization) in constitutive Piezo2-ChR2$^+$ (n=8), postnatal Piezo2-ChR2$^+$ (n=12), and Piezo2-Cre (n=6) mice evoked by blue light (462 nm) stimulation (2 Hz, 100 ms pulses) of the plantar surface of the hindpaws. Data for constitutive Piezo2-ChR2$^+$ and postnatal ChR2$^+$ is the same as that plotted in Fig. 1B. * denotes statistical difference between constitutive and virus-injected mice, ¥ denotes statistical difference between constitutive and control mice, and † denotes statistical difference between virus-injected and control (%withdrawal: ***P=0.0006, ¥¥¥¥P<0.0001, ††††P<0.0001; %Licking: **P=0.0092, ****P<0.0001, ¥¥¥P=0.0004, ¥¥¥¥P<0.0001, †P=0.041, ††P=0.0059, ††††P<0.0001; %guarding: ***P=0.0009 (0.151 mW/mm^2), ***P=0.0002 (0.516 mW/mm^2), ¥¥¥¥P<0.0001, †P=0.0228, ††††P<0.0001; %Flinching: ****P<0.0001, ¥¥¥¥P<0.0001, ††††P<0.0001; %jumping: ****P<0.0001, ¥¥¥¥P<0.0001, ††P=0.0019, ††††P<0.0001; %vocalization: ****P<0.0001, ¥¥¥¥P<0.0001, ††††P<0.0001. Two-way ANOVA Sidak's multiple comparison test).

Fig. S2. Mechanically activated currents in nociceptor subtypes of dorsal root ganglion neurons are Piezo2-dependent. Bar graph representing frequency distribution of dorsal root ganglion neurons into groups characterized by decay kinetics of mechanically activated currents; (**A**, **B**, and **D**) rapidly adapting (RA; τ_{inac} <10ms), intermediately adapting (IA; τ_{inac} 10 ms to 30 ms), and slowly adapting (SA; τ_{inac} >30 ms). Cells with no mechanically activated currents were grouped as non-responders (NR). Dorsal root ganglion neurons isolated from CGRP-GFP (**A**) and MRGPRD-GFP (**B**) mice. Mechanically activated currents were recorded from GFP positive neurons transfected with scrambled siRNA (CGRP: n=32, MRGPRD: n=50; 2 independent experiments each) or *Piezo2* siRNA (CGRP: n=33, MRGPRD: n=43; 2 independent experiments each). **P*=0.006, ***P*=0.002. (**C** and **D**) Mechanically activated currents in dorsal root ganglion neurons isolated from $Piezo2^{WT}$ (n=50, 2 independent experiments) and $Piezo2^{HoxB8}$ (n=48, 2 independent experiments) mice. Bars represent mean ± s.e.m **P*=0.043) (A) to (D) two tailed, non-parametric t-test.

Fig. S3. Response to mechanosensory stimulation of the eye (the blink reflex) is normal in $Piezo2^{HoxB8}$ mice. Percent response (blink) to stimulation of the cornea with von Frey filament at 0.008g, 0.04g and 0.4g in $Piezo2^{WT}$ (n=6) and $Piezo2^{HoxB8}$ (n=5) mice (n.s. not significant, one-way ANOVA Sidak's multiple comparison test). The blink reflex is normal in mice lacking PIEZO2 in caudal/upper thoracic sensory neurons.

Fig. S4. Response to innocuous and noxious stimuli in $Piezo2^{HoxB8}$ mice is not dependent on the sex of the animal. (**A** to **E**) Top panel is the same data plotted in Fig.2D to 2H. Bottom panel represents the data set in top panel but separated by sex of the animal. Females (f) and males (m) are represented as purple and orange circles, respectively (* denotes statistical difference between all mice, † denotes statistical difference between female mice, and ¥ denotes statistical difference between male mice, Mann Whitney non-parametric analysis). Bars represent mean. (**A**) Percent response (5 trials) to cotton swab stroke on the hindpaw in $Piezo2^{WT}$ (f=4,

m=11) and Piezo2^{HoxB8} (f=7, m=6) mice ($^{††}P$=0.003, $^{¥¥¥¥}P$<0.0001). (**B**) Number of bouts observed in response to an adhesive tape applied to the lower back of Piezo2^{WT} (f=4, m=7) and Piezo2^{HoxB8} (f=6, m=5) mice ($^{††}P$=0.0048, $^{¥¥}P$=0.0025). (**C**) Mechanical threshold measured in the range of 0.04g to 6g in Piezo2^{WT} (f=9, m=9) and Piezo2^{HoxB8} (f=11, m=6) mice ($^{††††}P$<0.0001, $^{¥¥¥}P$=0.0008). (**D**) Percent response (10 trials) to pinprick on hindpaw in Piezo2^{WT} (f=11, m=5) and Piezo2^{HoxB8} (f=10, m=5) mice ($^{††††}P$<0001, $^{¥}P$=0.0476). (**E**) Latency to response when an alligator clip (500g) is placed on base of the tail in Piezo2^{WT} (f=12, m=4) and Piezo2^{HoxB8} (f=9, m=5) mice ($^{†}P$=0.0184). Top panels in (A) to (E) *P=0.011, ***P<0.0001.

Fig. S5. Aδ-fiber and C-fiber mechanical and thermal thresholds in Piezo2^{HoxB8} mice. (**A**) Mean mechanical threshold of Aδ-fibers and C-fibers, in Piezo2^{WT} and Piezo2^{HoxB8} mice (**P=0.0095, n.s., Mann Whitney non-parametric test). (**B**) Mean heat thresholds (left) and mean spiking activity to a 4 second heat stimulus of 48°C (right) of thermoreceptive C-fibers in Piezo2^{WT} and Piezo2^{HoxB8} mice (n.s., Mann Whitney non-parametric test). (**C**), Mean spike activity of thermoreceptive C-fibers in response to continuous heat ramp from 32°C to 48°C (1°C/s) in Piezo2^{WT} and Piezo2^{HoxB8} mice. **B** and **C,** Piezo2^{WT}; n=9 and Piezo2^{HoxB8}; n=5 (n.s. between Piezo2^{WT} and Piezo2^{HoxB8} at all tested temperatures, two-way ANOVA with Bonferroni post-hoc analysis). n.s.: not significant.

Fig. S6. Capsaicin-induced thermal hyperalgesia is unaffected in Piezo2^{HoxB8} mice. (**A**) Measured paw size (mm) before and after 10 mins of capsaicin injection in Piezo2^{WT} (n=6, *P=0.015) and Piezo2^{HoxB8} (n=5, *P=0.03) mice. (**B**) Time taken to respond (by paw withdrawal, guarding or squeaking) when the hindpaw was submerged in a water bath maintained at 45ºC, before and after 5 mins of capsaicin injection in Piezo2^{WT} (n=6, **P=0.0022) and Piezo2^{HoxB8} mice (n=5, *P=0.015). Mann Whitney non-parametric test.

Fig. S7. Capsaicin-induced mechanical sensitization in Piezo2^{WT} and Piezo2^{HoxB8} mice. (**A**) Absolute mechanical threshold values in Piezo2^{WT} (n=9) and Piezo2^{HoxB8} (n=9) at baseline (before capsaicin injection) and 5, 15, and 30 minutes post capsaicin injection. ****P<0.0001, Two-way ANOVA with Holm-Sidak's multiple comparison. (**B**) Mechanical threshold values in Piezo2^{WT} (left) and Piezo2^{HoxB8} (right) mice at baseline (before vehicle or capsaicin injection) and 5, 15, and 30 minutes post vehicle or capsaicin injection.**P=0.001, ***P=0.0004, ****P<0.0001, Two-way ANOVA with Holm-Sidak's multiple comparison. (**C**) Allodynia score measured in response to brush stroke on hindpaw of Piezo2^{WT} (left) and Piezo2^{HoxB8} (right) mice at baseline (before vehicle or capsaicin injection) and 5, 15, and 30 minutes post vehicle or capsaicin injection. ***P=0.0008 (5 min), ***P=0.0002 (15 min), ***P=0.0003 (30 min), two-way ANOVA with Tukey's multiple comparison.

Fig. S8. Capsaicin-induced mechanical allodynia is compromised in Piezo2^{iAdv} mice. Absolute (left) and normalized (right) mechanical threshold at baseline (before capsaicin injection), 5, 15, and 30 minutes post capsaicin injection in Piezo2^{WT} (n=14) and Piezo2^{iAdv} (n=10) mice (**P=0.0027 (5 min, absolute), **P=0.0078 (15 min, absolute), ***P=0.0003 (30 min, absolute); ***P=0.0009 (5 min, normalized), *P=0.0115 (15 min, normalized), ***P=0.0003 (30 min, normalized), two-way ANOVA with Holm-Sidak's multiple comparison test).

Fig. S9. Spared nerve injury-induced mechanical sensitization in Piezo2^{WT} and Piezo2^{HoxB8} mice. (**A**) Absolute mechanical threshold values in Piezo2^{WT} (n=13) and Piezo2^{HoxB8} (n=13) mice on day 0 (before injury) and on day 7, 14, and 21 after spared nerve injury (****P<0.0001, two-way ANOVA with Holm-Sidak's multiple comparison test). (**B**) Absolute mechanical threshold (Piezo2^{WT}: n=13, **P=0.0073, ****P<0.0001; Piezo2^{HoxB8}: n=13), (**C**) mechanical allodynia (Piezo2^{WT}: n=17; Piezo2^{HoxB8}: n=15), and (**D**) pinprick hyperalgesia (Piezo2^{WT}: n=7, ****P<0.0001; Piezo2^{HoxB8}:n=7, *P=0.0408 (day 14), *P=0.0149 (day 21)) assessed on contralateral and ipsilateral paw of Piezo2^{WT} (left) and Piezo2^{HoxB8} (right) mice on day 0 (before injury) and on day

7, 14, and 21 after performing spared nerve injury (SNI). In (**C**) and (**D**) a score greater than 1 is indicative of stimulus induced painful behavior. Two-way ANOVA with Holm-Sidak's multiple comparison test.